Behavior of micropollutants in urban water systems, The University of Tokyo, Japan
November 26, 2015

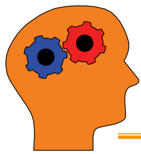
Antibiotic Resistance Characteristics in Antibiotic Polluted Aquatic Ecosystems

Yu ZHANG （张昱）

Research Center for Eco-Environmental Sciences,

Chinese Academy of Sciences

zhangyu@rcees.ac.cn

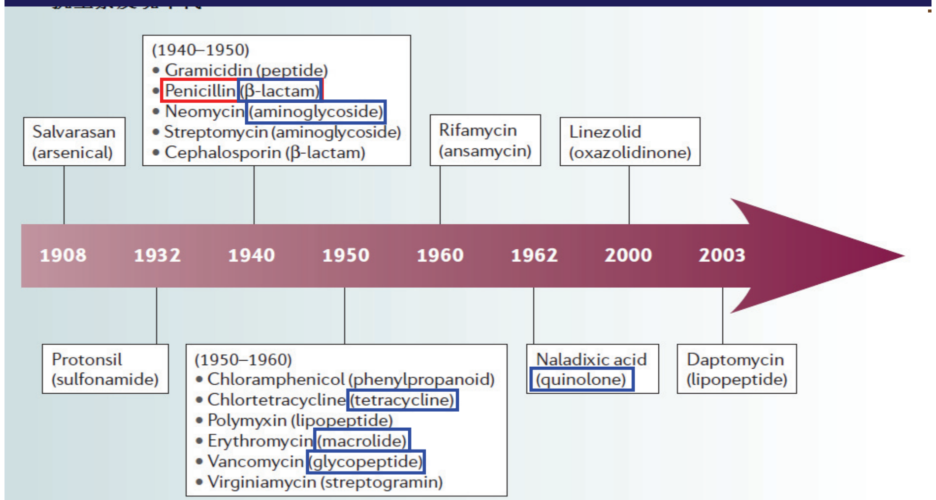


OUTLINE

- Background and Scientific Question
- Antibiotic Resistance Characteristics in Antibiotic Polluted Rivers
- Antibiotic Resistance Genes in WWTPs
- ARG Control Strategy

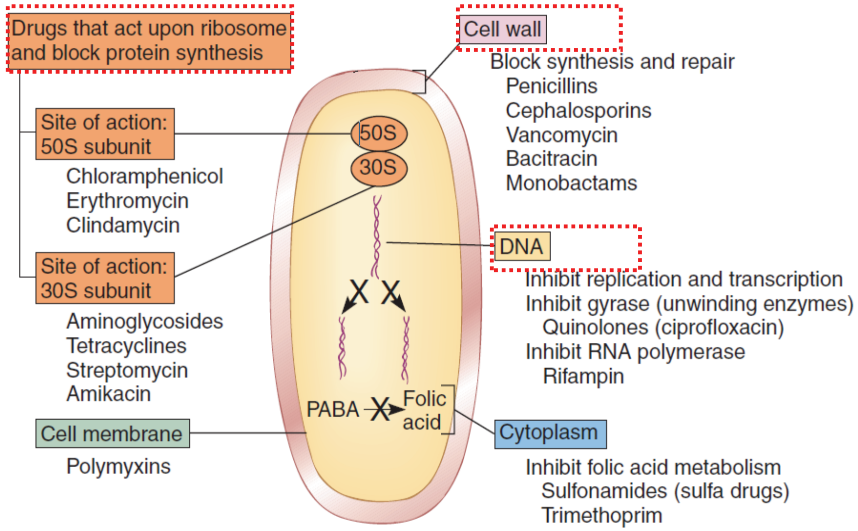
Two important centurial inventions

Story 1: Invention of Antibiotics



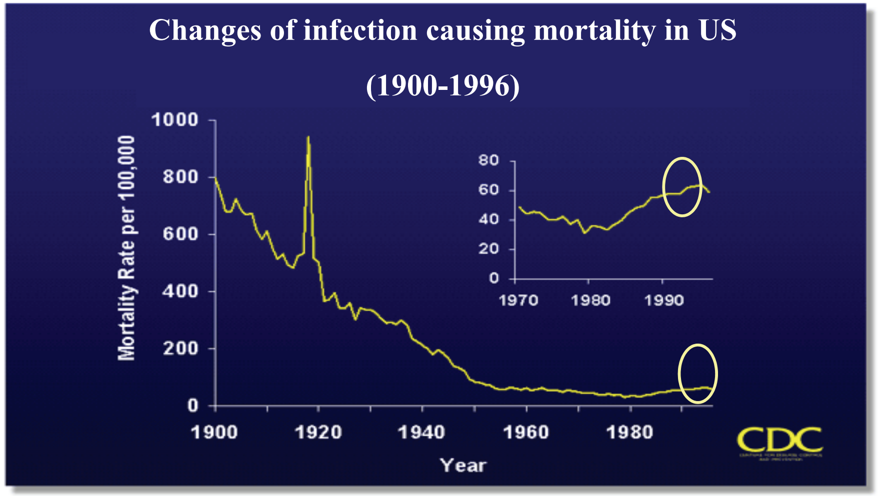
Since the discovery of the clinical application of penicillin, diverse kinds of antibiotics have been developed.

Inhibition Mechanism of Antibiotics for Bacteria



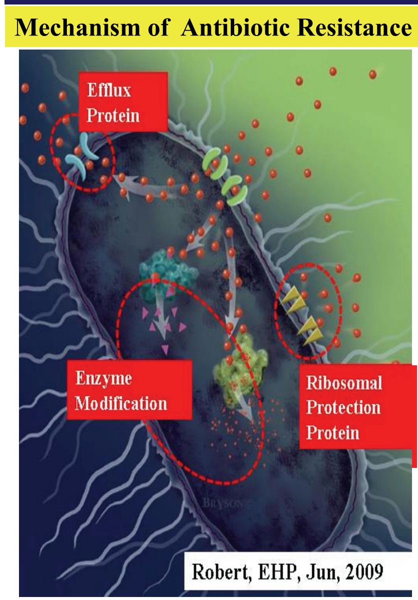
Antibiotics are designed to target Bacteria.

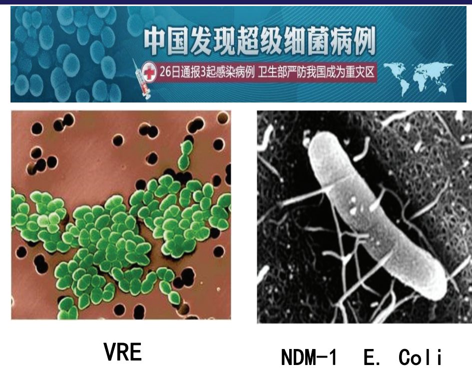
Impact of Antibiotic Resistance



Widespread antibiotic resistant bacteria have become one of the most serious challenges in clinical therapy

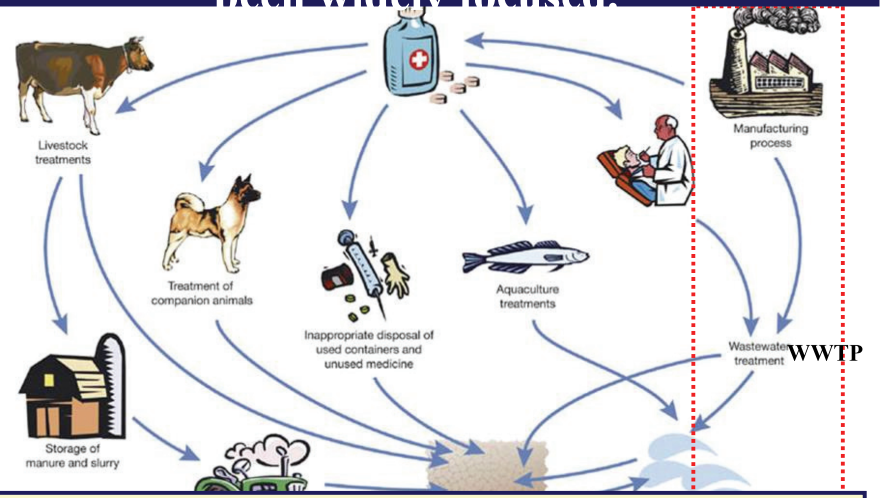
Super Bugs Becoming a Threat to Human





Antibiotic resistance genes (ARGs) have been considered as a new type of pollutants (ES&T, 2007).

Occurrence of antibiotics and ARGs have been widely focused.



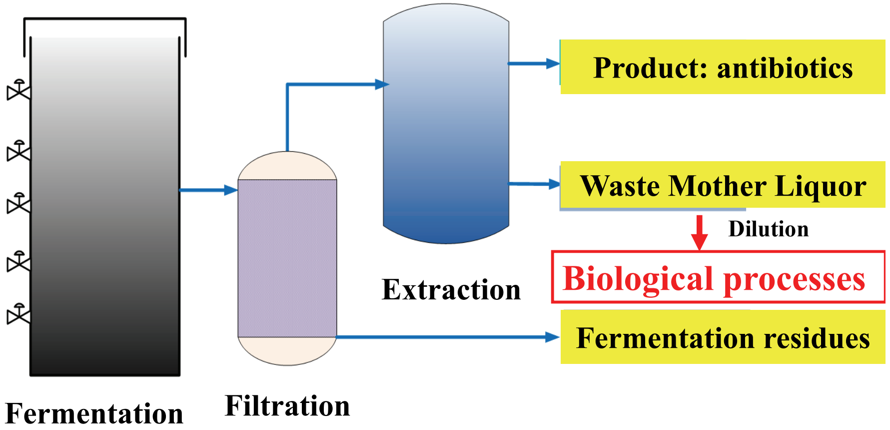
Antibiotic production process should be an important source for antibiotic pollution in environments.

Antibiotic Production in China

China is now one of the major antibiotic providers.

So the pollution of antibiotics in China is serious challenges.

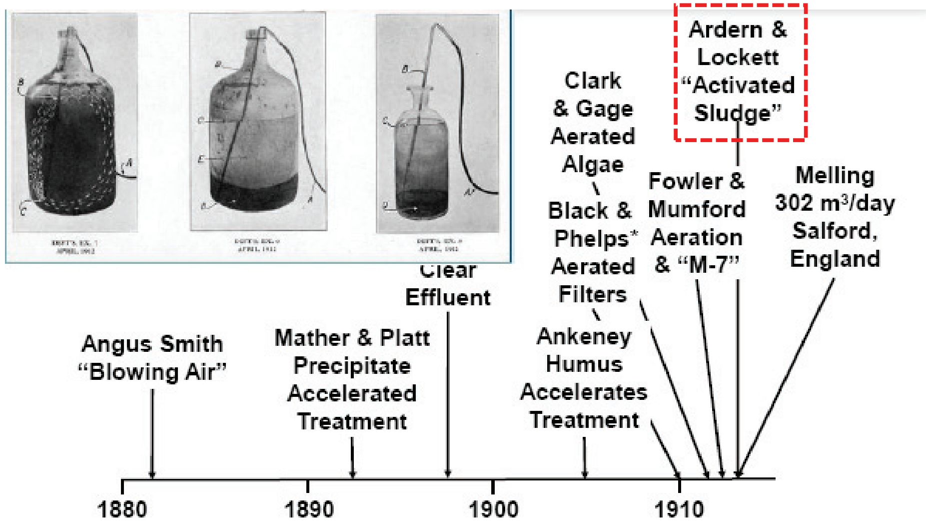
Pollutants Generated during Antibiotic Manufacturing



High residual antibiotics in APW (ETC, 2008; Water Res. 2009):

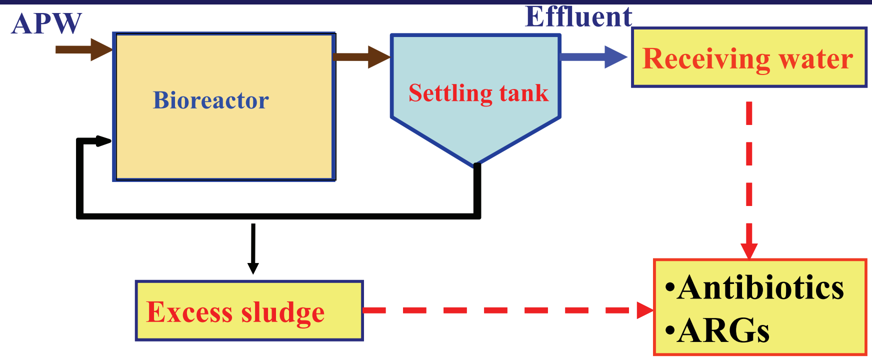
Mother liquor: 800-1100 mg/L;
Influent of biological treatment systems: several –several ten mg/L

Story 2: Invention of Activated Sludge



*Full-Scale, New York City

Hypothesis



Since antibiotics are designed to target bacteria, high antibiotic residues in wastewater could have significant impacts to antibiotic resistance of bacteria where bacteria normally play a dominant role in Activated Sludge.

Scientific Question

Activated Sludge

- How will high antibiotic concentrations in APW impact antibiotic resistance of bacteria in receiving ecosystems?
- How to control ARGs dissemination during antibiotics manufacturing?

Full-scale WWTPs: Antibiotic resistance genes in antibiotic production wastewater treatment systems

Sampling sites in WWTPs

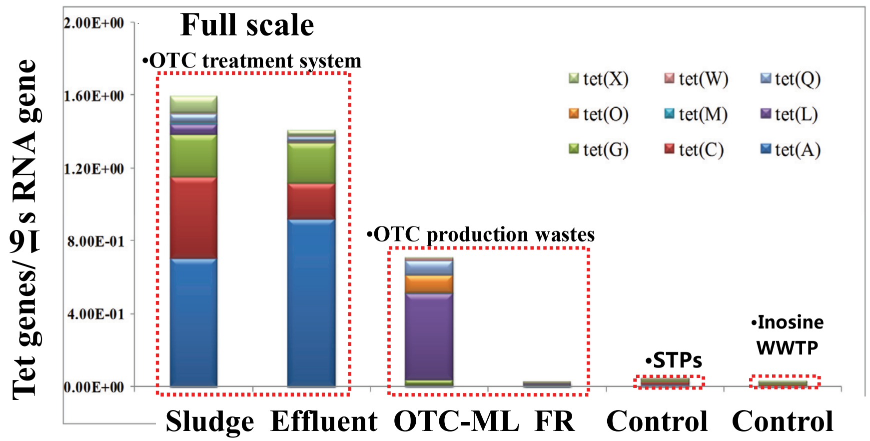
Tetracycline resistance (*tet*) genes

Time	Efflux	Ribosomal protection	Enzymatic (3)	Unknown[a]
	$n = 23$	$n = 11$	$n = 3$	$n = 1$
Listed in Ref. [1] Before 2001	$n = 18$ tet(A), tet(B), tet(C), tet(D), tet(E) tet(G), tet(H), tet(J), tet(V), tet(Y) tet(Z), tet(30), tet(31), tet(K), tet(L), tetA(P) otr(B), tcr3	$n = 9$ tet(M), tet(O), tet(S), tet(W), tet(Q), tet(T), otr(A), tetB(P)[b], tet	$n = 1$ tet(X)	$n = 1$ tet(U)
Not listed in Ref. [1] After 2001	$n = 5$ tet(33), tet(35)[d], tet(38), tet(39) otr(C)	$n = 2$ tet(32), tet(36)	$n = 2$ tet(34), tet(37)[c]	

tet(U) has been sequenced but does not appear to be related to either efflux or ribosomal protection proteins

Of 42 reported tet resistance gene in bacteria, at least 11 were found in AS bacteria

Changes of *tet* Genes during APW Treatment



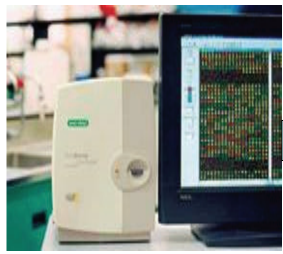
Tet genes: effluent and sludge > production wastes /MWTP/Control systems($p < 0.01$); *tet*(A), *tet*(C) and *tet*(G) were dominated.

Environ Sci Tech.2012,46:7551

ARGs and Antibiotic Concentration

- **The intensity of ARGs significantly increased with the increase of antbiotics（p<0.05）;**

Mantel analysis: 559 ARGs with antibiotic concentration



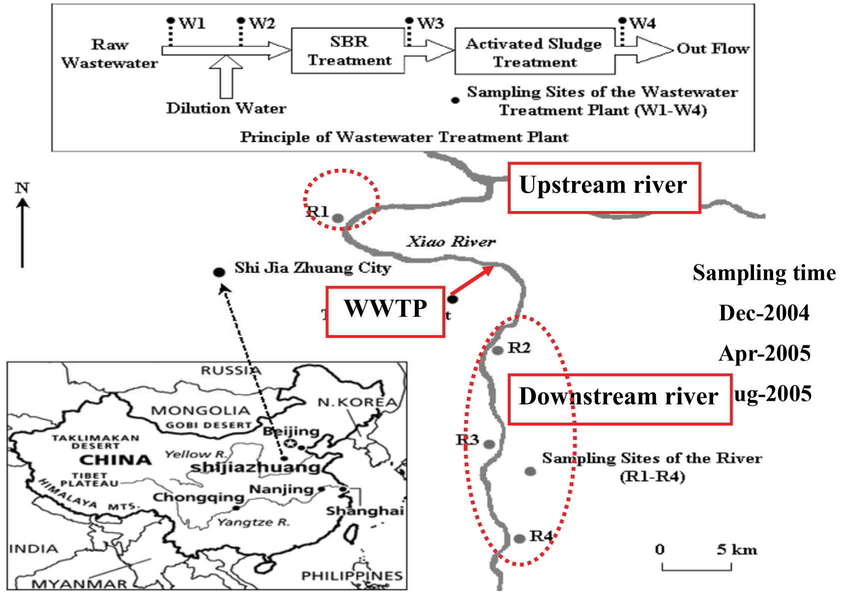
ARG tyoe	Correlation with antibiotics
Total antibiotic resistance genes	positively correlated
β-lactamase-A genes	positively correlated
β-lactamase-C (AmpC-type) genes	positively correlated
β-lactamase-D genes	positively correlated
MFS tansporters	positively correlated
SMR tansporters	positively correlated

***Water Res.* 2013,47:6298**

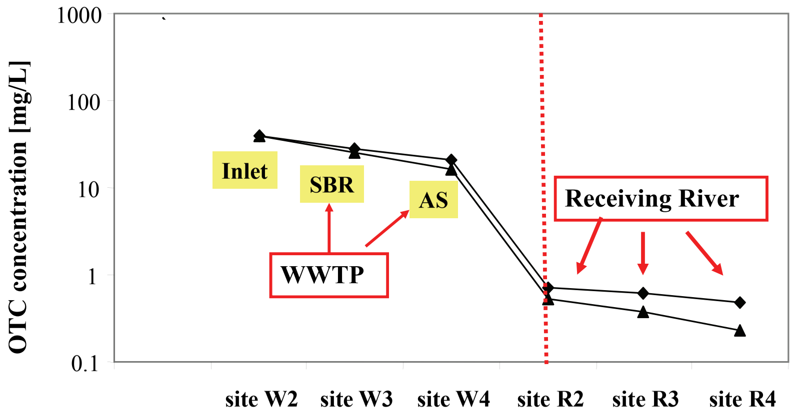
Field Study: Antibiotic Resistance Characteristics in Antibiotic Polluted Aquatic Ecosystem

Sampling sites in Shijiazhuang City, China

Sampling Map

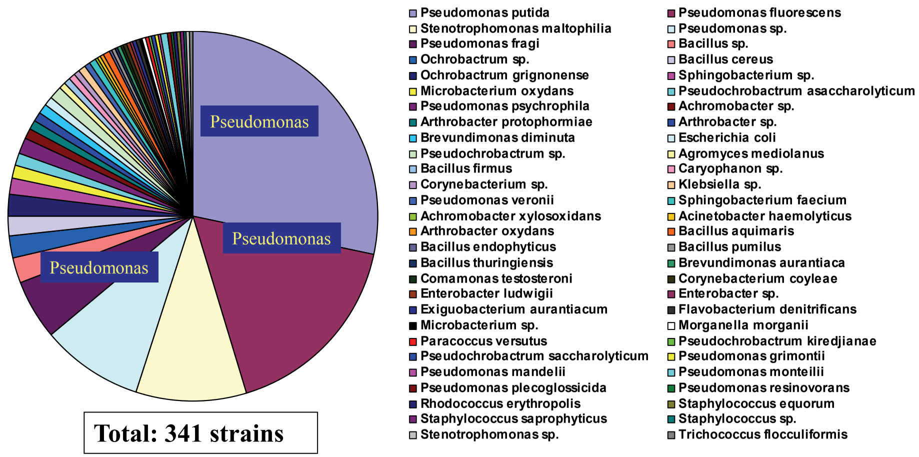


Changes of Antibiotic Concentration



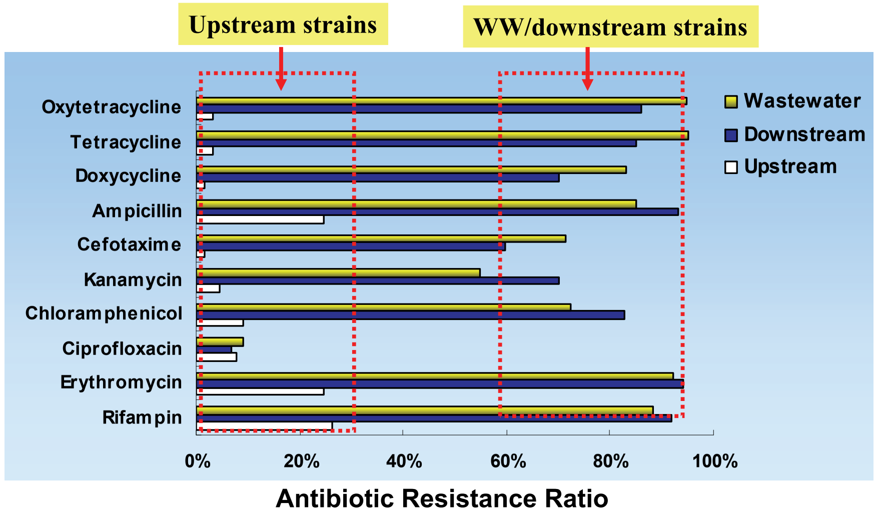
Biological treatment was not effective for the removal of OTC. High concentrations of OTC were detected in the receiving river.

Bacterial Isolates from WW and River



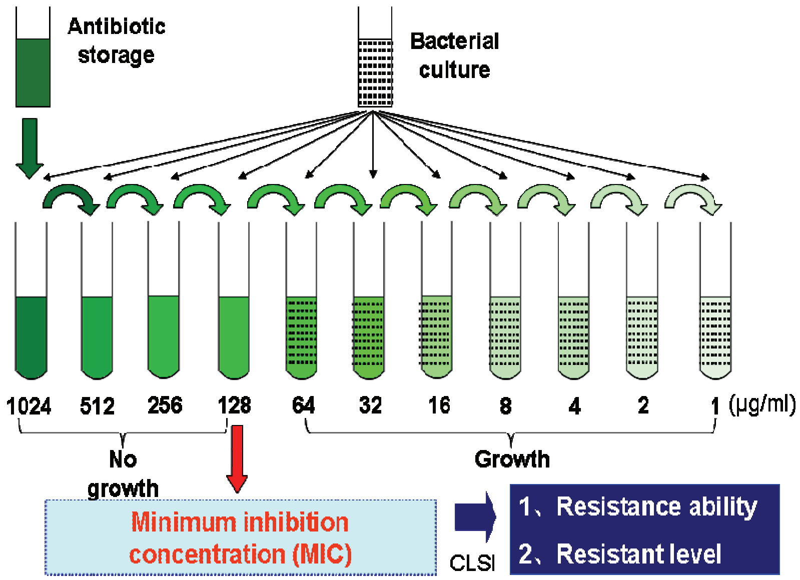
Bacterial isolates were acquired from the treated WW, upstream/downstream river using non-selective medium.

Resistance Characteristics: Resistance Ratio

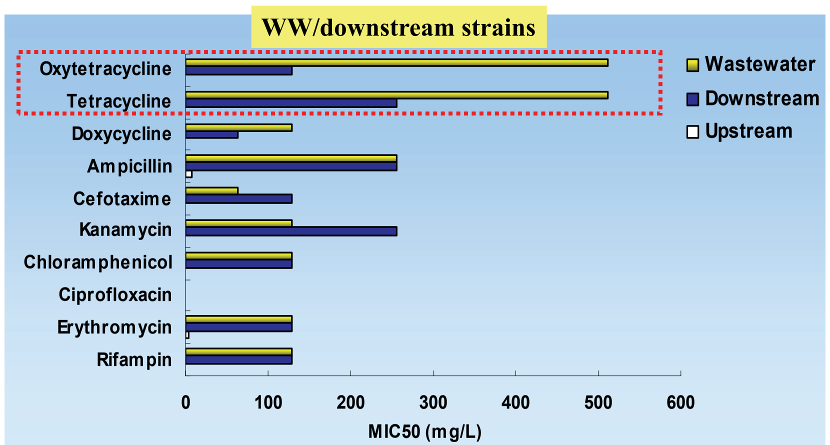


The treated WW and downstream river strains showed high resistance ratios in comparison with the upstream strains (EM,2009)

Method for MIC



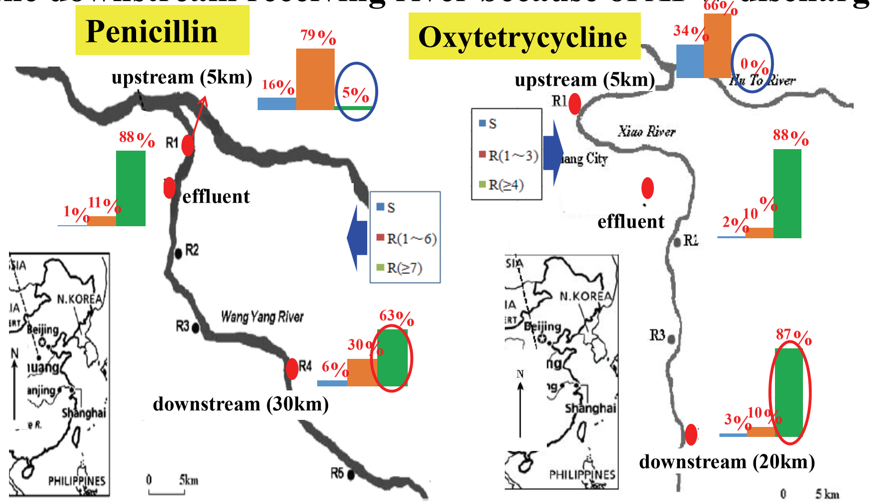
Resistance Characteristics:MIC50



The treated WW and downstream river bacteria showed much higher MIC_{50} values to different classes of antibiotics than the upstream strains. (WR, 2011)

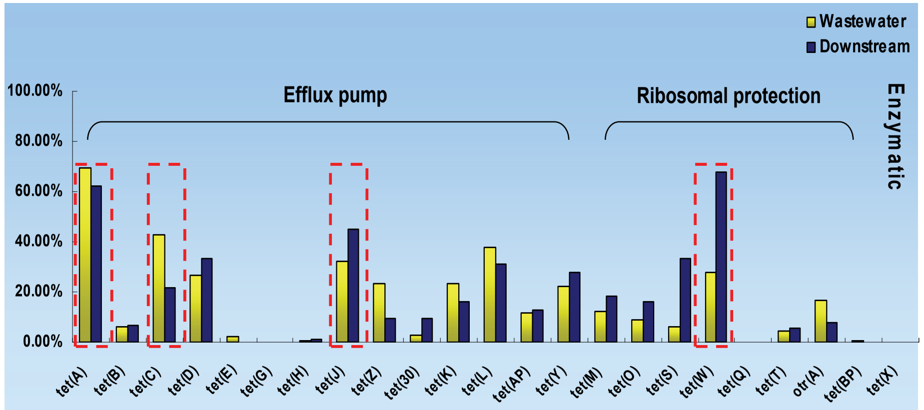
Multidrug Resistance of Bacterial Strain

Multidrug resistant bacteria were found to be increased in the downstream receiving river because of APW discharge.



Environ. Microbial, 2009,11:1506; Appl Environ Microb 2010,76:3444

Resistance Characteristics : ARG Types



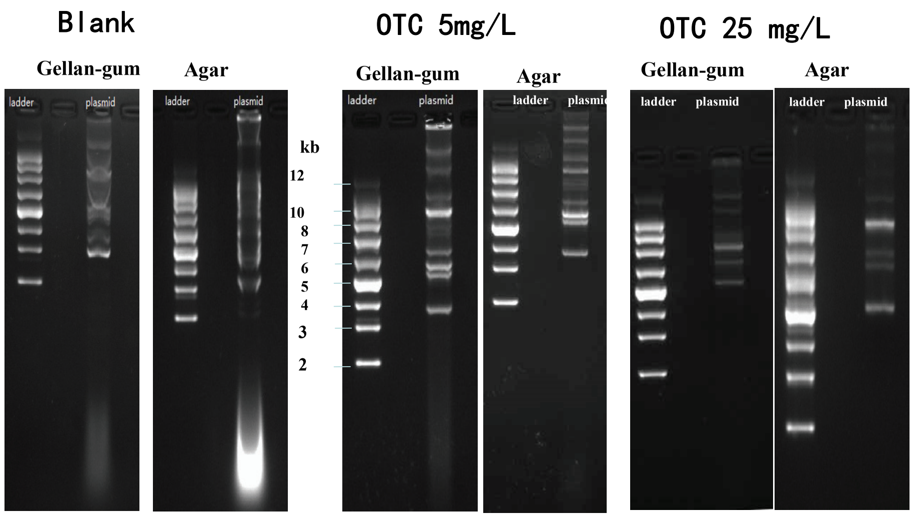
Treated WW and downstream river strains carried diverse *tet* resistance genes.
Tet(A)/(C)/(W)/(J) were dominant.

Hints from Field and Full-Scale Plant Studies

Hint 1: ARGs increased significantly during biological wastewater treatment.
Hint 2: Bacteria in treated wastewater and downstream river possessed strong resistance to multiple antibiotics.

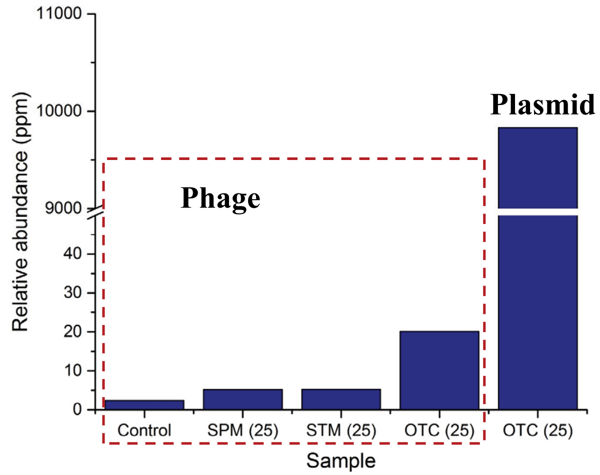
The above results were obtained mainly through field investigation. We further verified the results by conducting bench scale lab test.

Plasmid and phage DNA extracted



Lab Test -Aerobic Reactors

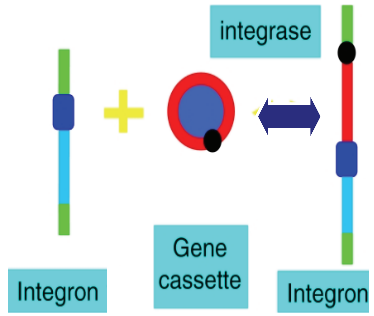
ARG Abundance from Plasmid and Phage



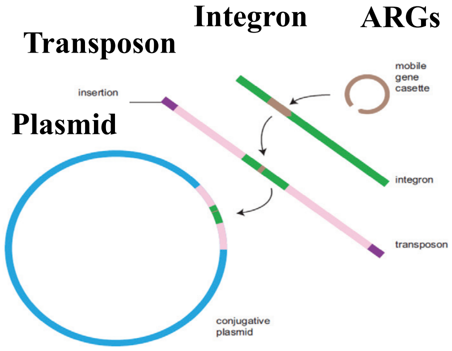
	Sample(mg/L)	ARGs abundance (ppm)
PLASMID	OTC(0)	3457
	OTC(5)	2385
	OTC (25)	9833
PHAGE	Control	2.43
	SPM (25)	5.17
	OTC(25)	20.16
	STM (25)	5.21

Metagenomic sequencing :
Plasmid DNA (Conjugation)

Conjugation :ARG cassettes capture by *Integron*

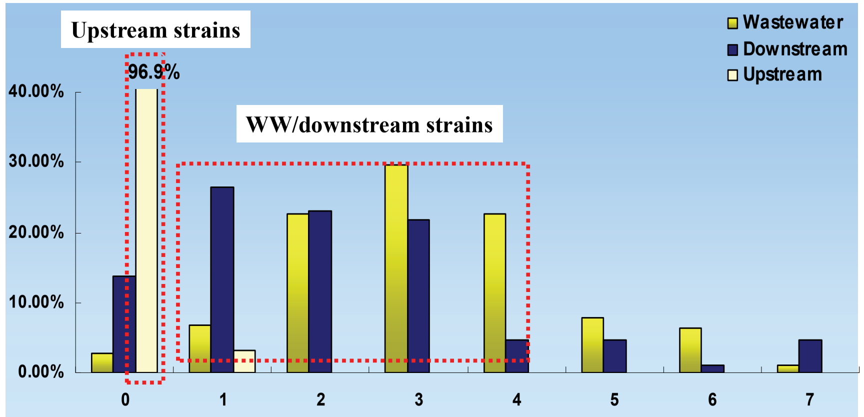


Cartoon of gene cassette capture by a bacterial integron.



ARGs were transferred from Integron to transposon or plasmid

Receiving River: Gene Cassettes in *intI 1*

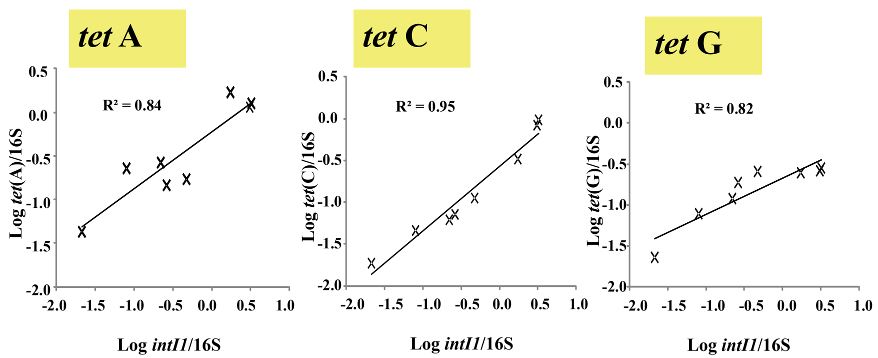


Number of gene cassettes *intI* 1 in bacterial strains

Most of WW and downstream river strains carried 1-4 gene cassettes in *intI* 1, while most of upstream river strains did not contain any gene cassettes.

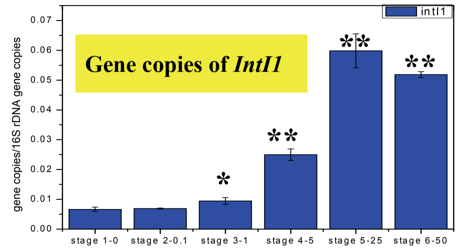
WWTP: Relationship between *tet* Genes and *intI* 1

Abundant ARGs in activated sludge: *tet*(A), *tet*(C) and *tet*(G)



***Tet*(A), *tet*(C) and *tet*(G) were highly correlated with *intI*1 (p<0.05). Class 1 integron might have played a role in increasing of *tet*(A)(C)(G) genes.**

Lab Test: Class I integron (*IntI*, HGT) Evaluation



***IntI* was significantly increased at 1 mg/L (qPCR), and ARGs were highly correlated with *intI*1 (p<0.05).**

Relationship of ARGs and *IntI1*

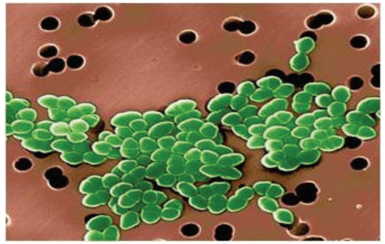
Genes	*IntI1*	*aac(3)-II*	*aacA4*	*aadA*	*aadE*	*aadB*	*aphA1*	*aphA2*	*strA*	*strB*
IntI1	1									
aac(3)-II	0.716	1								
aacA4	**.954****	0.562	1							
aadA	**.956****	**.813***	**.835***	1						
aadE	0.067	0.038	0.106	0.034	1					
aadB	0.656	0.746	0.413	**.826***	-0.261	1				
aphA1	-0.107	-0.055	0	-0.2	**.808***	-0.469	1			
aphA2	0.542	0.091	**.762***	0.325	0.019	-0.188	0.098	1		
strA	**.971****	0.667	**.897****	**.922****	0.046	0.687	-0.116	0.417	1	
strB	**.978****	0.649	**.931****	**.913****	0.16	0.611	-0.018	0.485	**.990****	1

**. Numbers denotes statistical significant correlation, p < 0.01 (Pearson coefficient, bivariate analysis).
*. Numbers denotes statistical significant correlation, p < 0.05 (Pearson coefficient, bivariate analysis).

Hints from Field, Full and Lab Scale Studies

Hint 1: ARGs increased significantly during biological wastewater treatment.
Hint 2: Bacteria in treated wastewater and downstream river possessed strong resistance to multiple antibiotics.
Hint 3: Plasmid and *Int*I may play important role in the occurrence of antibiotic resistance.

Problem: ARGs Discharge from WWTPs



VRE

NDM-1 *E. Coli*

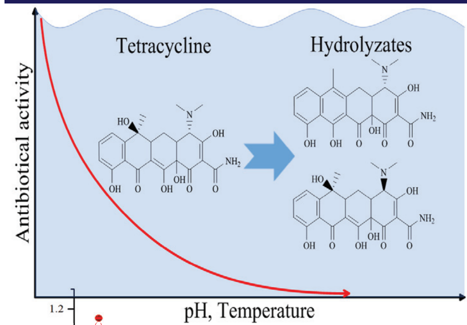
Measures should be taken to control the production of ARGs during treatment of antbiotic production wastewater.

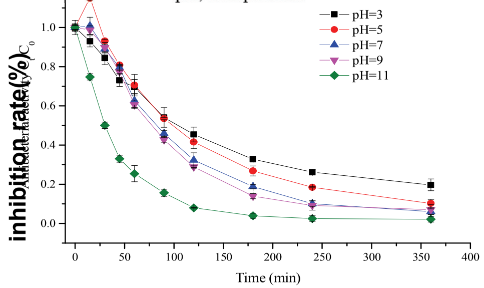
How to control the ARGs during wastewater treatment?

Development of abatement technologies

- Source control
- Thermophilic anaerobic digestion
- Yeast pre-treatment

Technology 1: Source Control





- Catalytic Hydrolysis
- Ozonation
- Fenton

After hydrolysis treatment or ozonation, antibiotic and antibiotic potency decreased.

Hydrolysis Poilt system

Technology 2: Treating OTC biosolid by AD

•Operation and stage of the continuous experiment

Stage	Feed	OTC conc. (mg/L)	Time (day)
1	35℃: Y ES:GBD ES=1:1 55℃:GBD ES	0	1～28
2	HY AS:GBD ES=1:1 (in TS)	0	28～76
3		40	76～125
4		200	125～166
5		1000	166～now (>230)

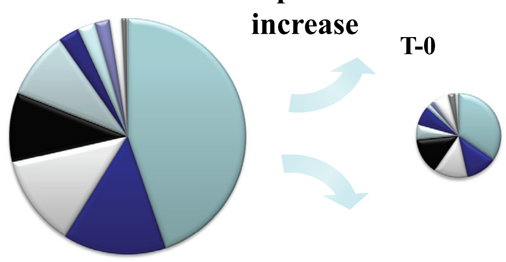
•SRT=20 days

Anaerobic Digestion Technologies to Remove ARGs

Yang, et al. EST, 2013

Mesophilic AD **Thermophilic AD**

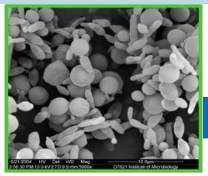
➢Illumina HiSeq 2500 (PE125);
➢Metagenomic sequencing data against the ARDB ;
➢BLASTX (E-value ≤ 1 × 10^{-5}; ≥90% amino acid identity; alignment length ≥25 amino acids)



In comparison with mesophilic anaerobic digestion, thermophilic anaerobic digestion has better ARGs removal efficiency . (Metagenomic sequencing)

Technology 3: Yeast pre-treatment

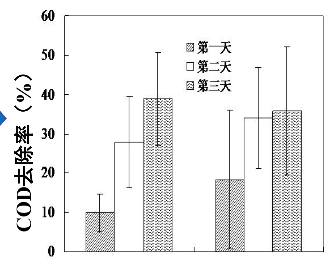
Fungi are normally not the target of antibiotics. Since key fungal functional genes were abundant in the presence of antibiotics, Fungi could be used for APW treatment without the ARG formation.



Candida tropicalis	热带假丝酵母
Candida boidinii	白假丝酵母
Pichia anomala	异常毕赤酵母
Candida lipolytica	解脂假丝酵母
Trichosporon asahii	阿萨希丝 孢酵母
Williopsis saturnus	土星拟威尔酵母

Yeasts isolated from polluted environments.

Yeast Poilt system in Wuxi city



COD removal

Application in Drug Industry

Technologies:

- ➢ Yeast pre-treatment;
- ➢ Anaerobic treatment for concentrated organic wastewater; excess sludge production reduced
- ➢ Advanced oxidation for antibiotics and ARGs removal

Besides the COD and NH_4^+ removal, antibiotics and ARGs abatement were also considered for safe discharge.

Acknowledgements

RCEES, CAS:

Prof. Min Yang

Dr. Zhe Tian;

Dr. Dong Li ;

Dr. Miaomiao Liu

Ms. Hong Zhang;

Ms. Yanhong Shi

Thanks to Prof. Furumai and Prof. Kurisu.

Thanks for the supports from NSFC and MOST, China.